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(71) Applicant *(for all designated States except US)*: DANISCO A/S [DK/DK]; Langebrogade 1, P.O. Box 17, DK-1001 Copenhagen K (DK).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ELSSER, Dieter** [DE/DE]; Lütte Weg 4, 25842 Bargum (DE). **MORGAN, Andrew, John** [GB/DK]; Fredensvej 1, DK-2950 Vedbæk (DK). **THOMAS, Linda, Valerie** [GB/GB]; 93 Monmouth Road, Dorchester, Dorset DT1 2DT (GB). **YU, Shukun** [SE/SE]; G. Hejdemansgatan 29, S-212 40 Malmoe (SE).

(74) Agents: **HARDING, Charles, Thomas** et al.; D Young & Co, 21 New Fetter Lane, London EC4A 1DA (GB).

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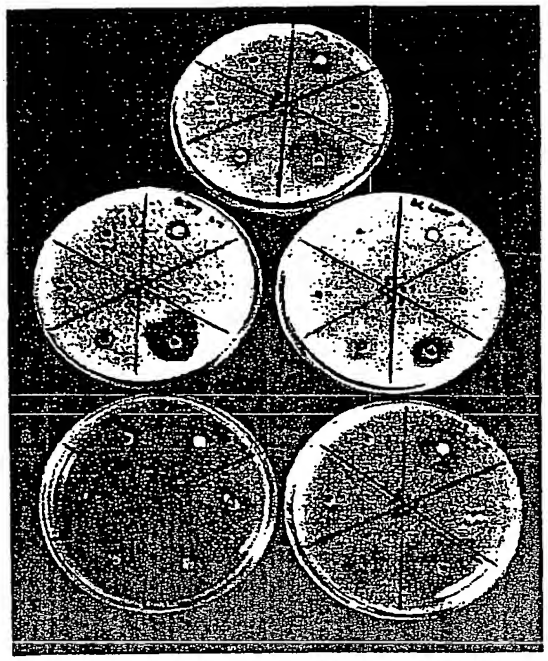
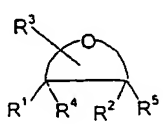
(54) Title: ANTIMICROBIAL AGENT





(I)

(57) Abstract: The present invention provides an antimicrobial composition comprising a cyclic compound having Formula I, wherein R^1 and R^2 are independently selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group; wherein R^3 is selected from -OH, =O, a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group, wherein R^4 and R^5 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound; and wherein said compound comprises at least one ester group. The invention further relates to a process for preventing and/or inhibiting the growth of, and/or killing, micro-organisms in a material, and the use of a cyclic compound having Formula I.

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ANTIMICROBIAL AGENT

The present invention relates to antimicrobial agents. More specifically, the invention relates to the antimicrobial activity of a series of anhydrofructose derivatives.

Food degradation from various sources is recognized in the literature and individual chemicals are known which will inhibit one aspect or another of degradation derived from a single source. Degradation, and the loss of colour or flavour of freshly cut plant parts are known to be caused by oxidation, enzymes, microbes, and metal ions. For example, acidulants are known to prevent microbial degradation by maintaining a relatively low pH environment but their effectiveness is only temporary.

Listeria monocytogenes is one example of an organism which can contaminate certain foodstuffs and which exhibits resistance to many physical and chemical treatments. *Listeria monocytogenes* is a gram-positive bacillus that causes serious infection, mainly in immunocompromised patients and newborn infants. Meningitis and bacteremia are the most frequent manifestations of listeriosis.

Bacillus cereus is another common cause of food poisoning. Two distinct clinical syndromes have been identified, the first having a short incubation period of about 4 hours, the second having an incubation period of about 17 hours. *B. cereus* food poisoning is initiated when the spore forms survive cooking and the contaminated food is allowed to reach temperatures that permit germination of the spore and elaboration of an enterotoxin.

Salmonella, of which there are over two thousand different strains, is a further cause of food poisoning in humans. *Salmonella* is a genus of rod-shaped Gram-negative *Enterobacteriaceae* that inhabit the intestine and cause infections such as gastroenteritis and typhoid. If invasive, they can cause enteric fevers (for example, typhoid caused by *Salmonella typhi,* or paratyphoid fever caused by *Salmonella paratyphi*). Other strains of *Salmonella* are associated with food poisoning (usually *Salmonella* Typhimurium, *Salmonella panama* or *Salmonella* Enteritidis, the latter notorious for the contamination

of poultry) and occasionally septicaemia in non-intestinal tissues.

It is well known in the art that *Salmonella* cannot propagate at pH values below 4.5. As a consequence, mildly acid products such as fine food and non-fermented meat products are especially susceptible to attack by *Salmonella*.

For meat products, nitrite is often used as a preservative. However, the addition of nitrite is restricted for toxological reasons (due to its acute toxicity, together with the dangers associated with nitrosamine formation). As a result, *Salmonella* is only inhibited at concentrations of nitrite beyond 1,000 ppm, which are far beyond legal limits.

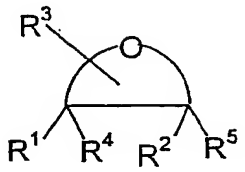
Instead, it has been shown that combinations of nitrite and sorbic acid can increase the effectiveness against *Salmonella* [Inhibition of *Salmonella* by Sodium Nitrite and Potassium Sorbate in Frankfurters, Journal of Food Science, 47, 1982, p. 1615 ff]. Inhibition has been observed at concentrations beyond 50 ppm of nitrite combined with 2600 ppm sorbic acid.

Other agents such as bacteriocins (Nisin) are unable to inhibit *Salmonella* in food, whereas benzoic acid is unsuitable because the inhibitory effect can only be observed in acid products. The inhibitory effect of phytogenic ingredients (or "natural substances") such as oil extracts from different spices, has also been tested, but again the concentrations required for achieving the inhibitory effect on *Salmonella* were too high and the sensorical influence on the food was too strong.

Thus, to date, the use of chemical substances has been severely limited because on the one hand they have to be safe from a toxicological view point, but on the other hand they must not influence the product sensorically.

The present invention seeks to alleviate the problems associated with prior art chemical substances and to provide new antimicrobial compositions based on anhydrofructose derivatives. In particular, the invention seeks to provide antimicrobial agents that are suitable for use in foodstuffs/feed.

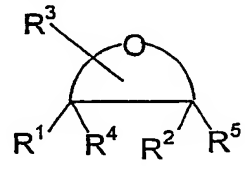
In a first aspect, the invention provides an antimicrobial composition comprising a cyclic compound having Formula I,



I

wherein R^1 and R^2 are independently selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group; wherein R^3 is selected from -OH, =O, a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group; wherein R^4 and R^5 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound; and wherein said compound comprises at least one ester group.

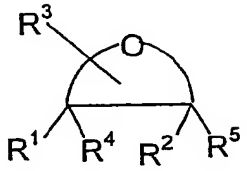
A second aspect of the invention provides a process for preventing and/or inhibiting the growth of, and/or killing, microorganisms in a material, the process comprising the step of contacting the material with a cyclic compound having Formula I,



I

wherein R^1 and R^2 are independently selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group; wherein R^3 is selected from -OH, =O, a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group; wherein R^4 and R^5 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound; and wherein said compound comprises at least one ester group.

In a third aspect, the invention relates to the use of a compound having Formula I,



I

wherein R^1 and R^2 are independently selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group; wherein R^3 is selected from -OH, =O, a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group; wherein R^4 and R^5 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound; and wherein said compound comprises at least one ester group; for preventing and/or inhibiting the growth of, and/or killing, microorganisms in a material.

It will be appreciated that by the term "ester group" it is meant a group of the formula X-C(O)O-Y wherein X and Y are hydrocarbyl groups.

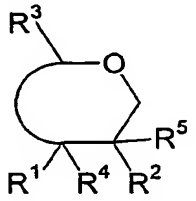
Preferably, the material is a foodstuff or feed. Thus, in a preferred aspect, the present invention relates to antimicrobial substances that are suitable for use in foodstuffs and/or feed to inhibit food poisoning and spoiling bacteria contained therein.

In another preferred embodiment, the material is a home product, a body care product or a cosmetic product, for example, a body lotion.

By way of definition, the term "antimicrobial" refers to a substance that kills or prevents or inhibits the growth or reproduction of microorganisms. Antimicrobials are generally classified according to the type of microorganism they are effective against. For example, antibacterial substances are effective against bacteria, antifungal substances are effective against fungi, including yeast, and antiviral substances are effective against viruses. Certain antimicrobials can be used internally, for example antibiotic medications, whereas other antimicrobials are for external use only, such as antiseptics.

As used herein, the term "hydrocarbyl group" means a group comprising at least C and H and may optionally comprise one or more other suitable substituents. Examples of such substituents may include halo-, alkoxy-, nitro-, hydroxy, carboxyl, epoxy, acrylic, hydrocarbon, N-acyl, or cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of substituents may form a cyclic group. If the hydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked *via* a suitable element or group. Thus, the hydrocarbyl group may contain hetero atoms. Suitable hetero atoms will be apparent to those skilled in the art and include, for instance, sulphur, nitrogen and oxygen.

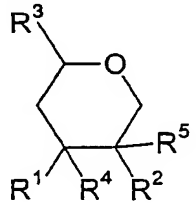
In a more preferred aspect, the cyclic compound of the invention is a compound having Formula II



II

wherein R^1, R^2, R^3, R^4, and R^5 are as defined hereinabove.

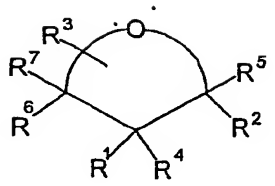
Preferably, the cyclic compound is a compound having Formula III



III

wherein R^1, R^2, R^3, R^4, and R^5 are as defined hereinabove.

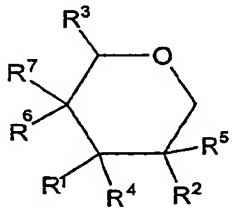
In one preferred embodiment, said cyclic compound is of Formula IV,



IV

wherein R^1 and R^2 are independently selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group; wherein R^3 is selected from -OH, =O, a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group; wherein R^4 and R^5 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound; wherein R^6 and R^7 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound; and wherein said compound comprises at least one ester group.

More preferably, said cyclic compound is of formula V,



V

wherein R^1, R^2, R^3, R^4, R^5, R^6 and R^7 are as defined hereinabove.

Preferably, R^1 is selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group.

Preferably, R^2 is selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group.

Preferably, R^3 is selected from a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group.

Even more preferably, R^3 is -OC(O)R', wherein R' is a H or a hydrocarbyl group.
Even more preferably, R^3 is -OC(O)R', wherein R' is a hydrocarbyl group.

In one preferred embodiment, R^3 is -OC(O)R', wherein R' is R'' group.

Preferably, R' and/or R'' is a branched or unbranched, substituted or unsubstituted alkyl group.

More preferably, R' and/or R'' is $(CH_2)_pCH_3$, wherein p is from 1 to 24.

Even more preferably, R' and/or R'' is a C_8 alkyl group.

In an another preferred embodiment, R' and/or R'' is a C_{12} alkyl group.

In an another preferred embodiment, R' and/or R'' is a C_{16} or a C_{18} alkyl group.

In one preferred embodiment of the invention, R^3 is of the formula $-(CH_2)_n-OC(O)-(CH_2)_pCH_3$, wherein n and p are each independently from 1 to 24.

More preferably, R^3 is of the formula $-(CH_2)_n-OC(O)-(CH_2)_7CH_3$, wherein n is from 1 to 24, preferably from 1 to 20, preferably from 1 to 10, preferably from 1 to 5, or preferably 1, 2, or 3.

In an alternative preferred embodiment, R^3 is of the formula $-(CH_2)_n-OC(O)-(CH_2)_{11}CH_3$, wherein n is from 1 to 24, preferably from 1 to 20, preferably from 1 to 10, preferably from 1 to 5, or preferably 1, 2, or 3.

In one preferred embodiment, R^4 is selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group.

In a particularly preferred embodiment, R^4 is selected from a hydrocarbyl group, H, OH, and =O.

In one preferred embodiment, R^5 is selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group.

In a particularly preferred embodiment, R^5 is selected from a hydrocarbyl group, H, OH, and =O.

In one preferred embodiment, R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound.

In one especially preferred embodiment, the compound is esterified anhydrofructose wherein at least one OH group of anhydrofructose is esterified to form a -OC(O)R''' group, wherein R''' is a hydrocarbyl group.

Preferably, R''' is a branched or unbranched, substituted or unsubstituted alkyl group.

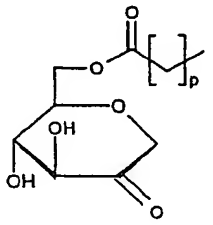
Even more preferably, R''' is $(CH_2)_pCH_3$, wherein p is from 1 to 24,

More preferably still, R''' is a C_8 alkyl group.

In an alternative preferred embodiment, R''' is a C_{12} alkyl group.

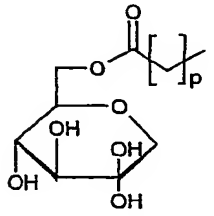
In another preferred embodiment, R''' is a C_{16} or a C_{18} alkyl group

In one preferred embodiment of the invention, the cyclic compound is of the formula:



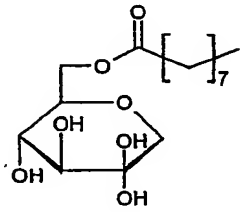
p = 1-24

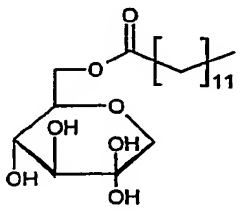
In one preferred embodiment of the invention, cyclic compound is of the formula:



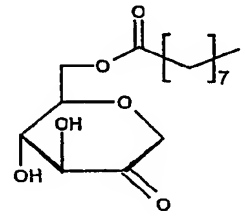
p = 1-24.

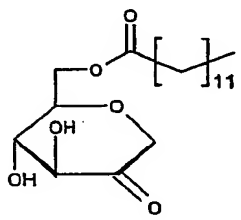
More preferably, the cyclic compound is selected from the following:





More preferably, the cyclic compound is selected from the following:

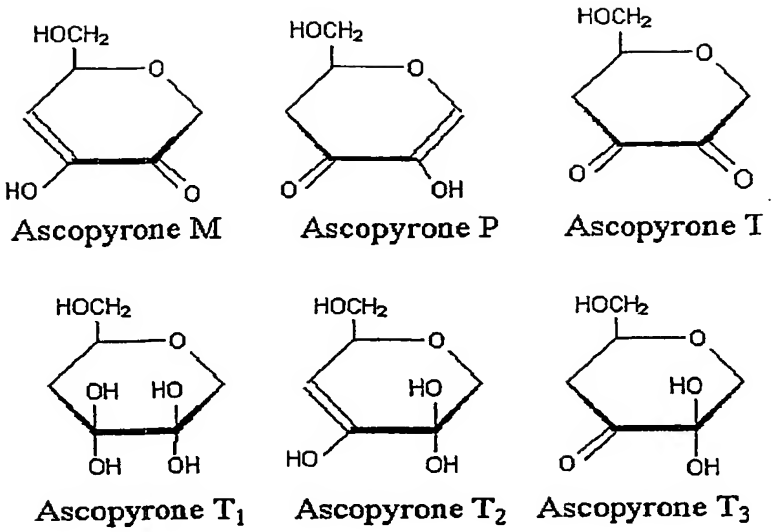




Preferably, the compound of the invention is a derivative of Ascopyrone P, Ascopyrone M, Ascopyrone T, Ascopyrone T_1, Ascopyrone T_2, Ascopyrone T_3, and mixtures thereof.

Even more preferably, the compound of the invention is selected from esterfied Ascopyrone P, esterfied Ascopyrone M, esterfied Ascopyrone T, esterfied Ascopyrone T_1, esterfied Ascopyrone T_2, esterfied Ascopyrone T_3, and mixtures thereof.

The structures of Ascopyrone P, Ascopyrone M, Ascopyrone T, Ascopyrone T_1, Ascopyrone T_2 and Ascopyrone T_3 are shown below.



Ascopyrone is a known compound. In 1978 and 1981, a group of American scientists prepared Ascopyrone P by pyrolysis of amylopectin, amylose and cellulose at the Wood Chemistry laboratory in Montana, with the intention of using Ascopyrone P as a starting material for organic synthesis [Shafizadeh, F., Furneaux R.H., Stevenson, T.T., and Cochran, T.G., 1,5-Anhydro-4-deoxy-D-*glycero*-hex-1-en-3-ulose and other pyrolysis products of cellulose, Carbohydr. Res. 67(1978): 433-447; Stevenson, T.T., Stenkmap, R.E., Jensen, L.H., Cochran, T.T., Shafizadeh, F., and Furneaux R.H., The crystal structure of 1,5-anhydro-4-deoxy-D-*glycero*-hex-1-en-3-ulose, Carbohydr. Res. 90(1981): 319-325]. They characterized Ascopyrone P by, for example, ^{1}H and ^{13}C NMR, and IR spectroscopy techniques. A 3-dimensional structure of Ascopyrone P was provided. The yield of Ascopyrone P obtained by pyrolysis was under 3% and complicated separation methods had to be used.

The natural occurrence of Ascopyrone P in some species of very scarcely studied fungi collected from the Alps has been taught [M.-A. Baute, G. Deffieux, J. Vercauteren, R. Baute, and Badoc A., Enzymatic activity degrading 1,4-α-glucans to Ascopyrones P and T in *Pezizales* ad *Tuberales*, *Phytochemistry*, 33 (1993): 41-45]. The occurrence of Ascopyrone P in fungi immediately prompted the hypothesis that Ascopyrone P would act as an antibiotic. However, Ascopyrone P did not function satisfactorily as an antibiotic in the disclosed tests.

Ascopyrone P and Ascopyrone T can be produced enzymatically from 1,5-anhydro-D-fructose using cell-free extract prepared from the fungi of the order *Pezizales*, such as *Plicaria leiocarpa* and *Anthracobia melaloma*, and the order of Tuberales, such as, *Tuber melanosporum*. Ascopyrone T_1 is the dihydrate form of Ascopyrone T, whereas Ascopyrone T_2 and T_3 are the tautomeric monohydrate forms of Ascopyrone T.

Ascopyrone M can be produced from 1,5-anhydro-D-fructose by EDTA-sensitive dehydratases isolated from the fungi Morels, such as *Morchella vulgaris*, *Gyromitres*, *pezizes*, such as *Peziza echinospora*.

Ascopyrone M, P and T can also be produced chemically by treating 1,5-anhydro-D-fructose with alkali under mild conditions [Studies on the degradation of some pentoses and of 1,5-anhydro-D-fructose, the product of the starch-degrading enzyme a-1,4-glucan lyase; Thesis, Ahmad, T., The Swedish University of Agricultural Sciences, Sweden, 1995].

When the compound of the present invention is prepared by chemical means, it may be prepared in accordance with one of the following methods:

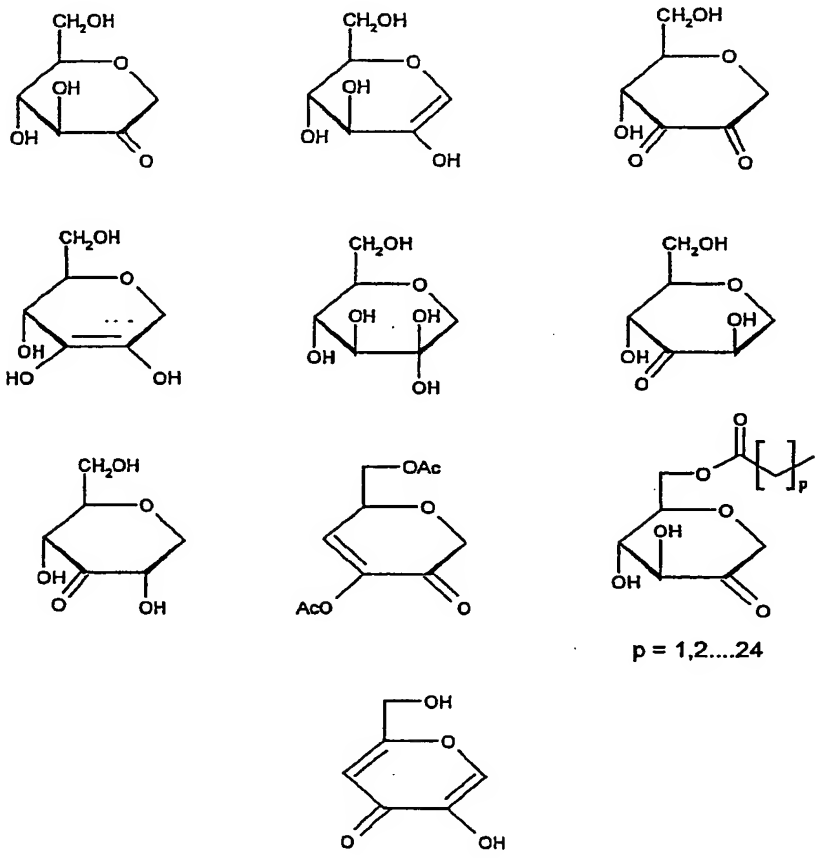
(1) Ascopyrone P may be produced by treating 1,5-anhydro-D-fructose with non-aqueous acid at elevated temperature, for example at 70 °C.

(2) Ascopyrones (for example, Ascopyrone P, T and M) may be produced from 1,5-anhydro-D-fructose by alkaline treatment according to Ahmad, T., 1995.

The structures of all ascopyrones produced were confirmed by NMR techniques.

Preferably, the compound of the present invention is prepared by enzymatic means as disclosed in M.-A. Baute *et al*, [*Phytochemistry*, 33 (1993): 41-45). For example ascopyrones (such as, Ascopyrone P, T and M) may be produced from 1,5-anhydro-D-fructose using enzymatic methods as disclosed in M.-A. Baute *et al*.

In a particularly preferred embodiment, the compound is selected from the following:



or an esterified derivative thereof.

In a preferred embodiment, the cyclic compound having formula I has an antimicrobial effect against gram positive bacteria and yeasts.

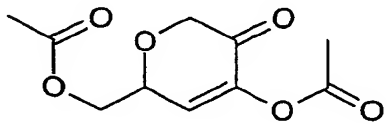
Preferably, the cyclic compound having formula I has an antimicrobial effect against a microorganism selected from *Listeria, Salmonella, Bacillus, Saccharomyces, Pseudomonas, Clostridium, Lactobacillus, Brochothrix, Micrococcus, Yersinia, Enterobacter* and *Zygosaccharomyces, Staphylococcus, Escherichia.*

Even more preferably, the cyclic compound having formula I has an antimicrobial effect against a microorganism selected from *Listeria monocytogenes, E. coli, Staphylococcus*

aureus, Listeria innocua, Salmonella Typhimurium, *Salmonella* sp., *Bacillus cereus, Bacillus subtilis, Saccharomyces cerevisiae, Saccharomyces cerevisiae* var. *paradoxus, Saccharomyces carlsbergensis, Pseudomonas fluorescens, Clostridium sporogenes, Lactobacillus sake, Brochothrix thermosphacta, Micrococcus luteus, Yersinia enterocolitica, Enterobacter aerogenes* and *Zygosaccharomyces bailii.*

Even more preferably, the cyclic compound having formula I has an antimicrobial effect against a micro-organism selected from *Listeria monocytogenes, E. coli, Bacillus cereus, Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Pseudomonas fluorescens, Clostridium sporogenes, Lactobacillus sake, Brochothrix thermosphacta* and *Micrococcus luteus.*

In a highly preferred aspect a derivative of the compound of formula I is a compound of the formula



This compound (3,6-di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-3-enopyranose-2-ulose) may be prepared in accordance with the teaching of Andersen *et al.* (1998), Structure of 1,5-anhydro-D-fructose: X-ray analysis of crystalline acetylated dimeric forms, J. Carbohydr. Chem. 17: 1027-1035.

The aspect of the present invention wherein the derivative of the compound of formula I is an ester is particularly preferred because the compound may be lipophilic and/or may have both hydrophobic and hydrophilic properties. When the compound has both hydrophobic and hydrophilic properties the compound readily resides at a water/oil interface of an emulsion.

The residence of the compound at a water/oil interface of an emulsion may allow it to act as an emulsifier. Thus the present invention may further provide compounds having a

dual functional effect. The compounds may act both as an antimicrobial and as an emulsifier.

Many of the compounds of the present invention can be derived from 1,5-anhydrofructose. 1,5-Anhydrofructose is monoketo sugar found in bacteria, red algae, fungi and mammals. In red algae and fungi 1,5-anhydrofructose is produced by the action of α-1,4-glucan lyase [EC 4.2.2.13] from floridean starch and glycogen, respectively.

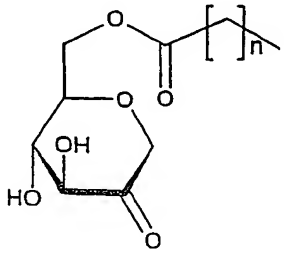
When the compound of the present invention is prepared from 1,5-anhydro-D-fructose, preferably the 1,5-anhydro-D-fructose is prepared in accordance with GB-A-2296717. In other words, preferably the 1,5-anhydro-D-fructose is prepared by a method comprising treating an α-1,4-glucan with the enzyme α-1,4-glucan lyase characterised in that enzyme is used in substantially pure form.

Preferably, the cyclic compound of the invention comprises a five or a six membered ring.

The compounds of the present invention comprise at least one ester group. Thus, as used herein the term "ester" includes mono-, di-, tri- and poly-esters.

In a preferred aspect the compound of formula I is a diester wherein the R^1 substituent is an -OH group and wherein the ester linkages are formed from the -OH group of the R^4 substituent and from the -OH group of the R^3 substituent.

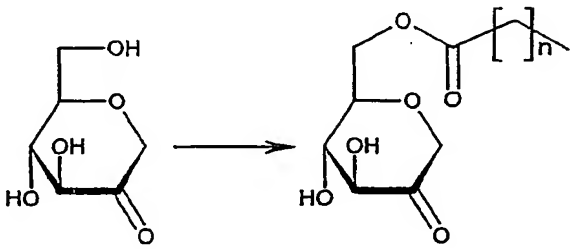
As mentioned above, in a particularly preferred embodiment of the invention, the compound is 6-*O*-acyl-1,5-anhydro-D-fructose, as represented below.



The preparation of 6-*O*-acyl-1,5-anhydro-D-fructose may be addressed by a chemical

approach or by an enzymatic approach, in accordance with the methods detailed in WO 00/56745.

The chemical approach may comprise the following reaction to synthesise C_{12} esters of anhydrofructose:



The reaction is carried out with lauroyl chloride and pyridine. The acylation sites were assigned through derivatisation of NH_2OR followed by separation and NMR of the products. The products were found to be

50% 6-*O*-acyl-1,5-anhydro-D-fructose

11% 3-*O*-acyl-1,5-anhydro-D-fructose

A similar method may be used to prepare other ester derivatives of anhydrofructose.

The enzymatic approach to prepare 6-*O*-acyl-1,5-anhydro-D-fructose may comprise the use of lipases and proteases. In aqueous solution lipases and proteases cleave ester linkages. Lipases are sugar specific and proteases fatty acid specific. However, *Synthesis* 1990, 112-115 discloses that lipases and proteases in non-aqueous solution offer a reversal of activity, and form ester bonds. Thus lipases and proteases in non-aqueous solution may be used in the preparation of a compound in accordance with the present invention.

In accordance with *J. Chem. Soc. Perkin Trans.* I, 1995, 2203-2222 lipases were screened to identify suitable lipases for the preparation of compounds in accordance with the present invention. Screening with pyridine identified *Candida antarctica, Pseudomonas cepacia, Pseudomonas fluorescens*, and hog pancreas. Screening with tBuOH:pyridine 2:1 identified *Candida antarctica, Candida cylindracea,*

Pseudomonas cepacia, Pseudomonas fluorescens, hog pancreas.

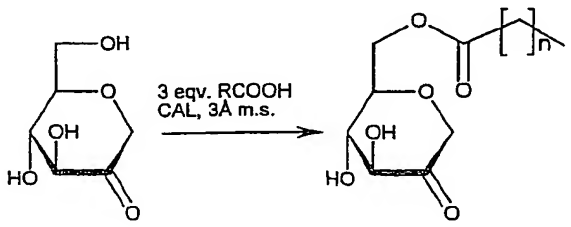
Thus preferably the compound in accordance with the present invention is prepared with a lipase obtained from *Candida antarctica, Pseudomonas cepacia, Pseudomonas fluorescens*, hog pancreas, or *Candida cylindracea.*

Preferably the compound in accordance with the present invention is prepared with lipase from *Candida antarctica. Candida antarctica* may be obtained from Novo Nodisk A/S, Denmark under the name Novozym 435.

The enzymatic approach was demonstrated by the enzymatic acylation of 1,5-anhydro-D-fructose with lauric acid to form 6-*O*-acyl-1,5-anhydro-D-fructose.

Lauric acid (mol/mol)	Solvent	3 Å molecular sieve. (w/w)	Temperature (oC)	Reaction time (h)	Conversion
1	tert-BuOH	-	40	24	21 %
1	tert-BuOH	1 (powd.)	40	24	56 %
1	tert-BuOH	1 (powd.)	40	72	62 %
1	acetone	1 (powd.)	20	24	55 %
1	tert-BuOH	5	45	24	56 %
1	tert-BuOH	10	45	24	61 %
1	tert-BuOH	20	45	24	66 %
3	tert-BuOH	20	45	24	73 %
3	tert-BuOH	20 (powd.)	45	24	78 %
3	tert-BuOH	20 (powd.)	45	48	quantitative
3	acetone	20	20	72	quantitative

The chemical approach may comprise the quantitative conversion with lauric, palmitic and stearic acid of 1,5-anhydro-D-fructose to 6-O-acyl-1,5-anhydro-D-fructose as follows:



The reaction forms a composition comprising monomer ketone/dimer type 1/dimer type 2 - 1:3:1. The mixture may be purified by chromatography on silica to give approximately

70% yield.

The cyclic compound of the invention may be used alone, or in combination with other components, for example, one or more preservatives, one or more chelators (such as EDTA sodium salt, polyphosphate or citrate) and/or one or more antioxidants (such as ascorbate, isoascorbate, ascorbate palmitate, BHA or BHT).

By way of definition, in the broadest sense, the term "preservative" is intended to encompass all substances which inhibit the development of, or kill, micro-oganisms. In a narrower sense, it is generally understood that preservatives are used in concentrations of 0.5 % or less. Food additives which are allowed to be used as preservatives are listed in the Regulation No. 95/2/EG of the European Parliament and Council of 20 February 1995, relating to food additives other than colouring agents and sweeteners.

Typical food preservatives permitted in the EU which are suitable for use in combination with the compounds of the invention include sorbic acid, benzoic acid, PHB ester (p-hydroxybenzoate), and sulphur dioxide. The mode of action of these preservatives, together with their range of effects are listed below.

Sorbic Acid (E200 to 203):
Mode of action: inhibits different enzymes in the cells of the microorganisms.
Range of effects: mainly against yeasts and moulds as well as catalase-positive bacteria. Catalase-negative bacteria as well as lactic acid bacteria and clostridia are not inhibited.
Effective concentration: 500 - 3000 ppm.
Permitted maximum quantities in food: up to 2000 ppm in potato dough, processed cheese, packed bread, fine bakery products, emulsified sauces etc.

Benzoic Acid (E210 to 213):
Mode of action: inhibits exchange of oxygen through the cellular membrane and affects the enzymatic structure.
Range of effects: for acid products only, up to approx. pH 4.5; inhibits yeasts and moulds, restricted inhibition of bacteria (no, or only very little, inhibition of lactic acid

bacteria and clostridia).

Permitted maximum quantities in food: 500 ppm in aspic, fruit preparations, marmalades etc.

PHB Ester (p-hydroxybenzoate) (E214 to 219)

Mode of action: damages the bacterial membrane because of the surface activity, poisonous to protoplasm because of protein denaturation.

Range of effects: mainly inhibits yeasts and fungi, but also Gram-positive bacteria in a pH range between 3.0 and 8.0.

Effective concentration: sensorical influence at concentrations beyond approx. 0.08 %.

Sulphur Dioxide (E220 to 224; E 226 to 227)

Mode of action: depends on pH to a great extent, in practice it is only effective at acidic pH values (< 4,0). Very complex mechanisms.

Range of effects: mainly antibacterial, above all against Gram-negative, aerobic bacteria.

Effective concentrations: 250 - 500 ppm for inhibition of aerobic, Gram-negative bacteria, 800 - 2000 ppm against Gram-positive bacteria, yeasts, and moulds.

Permitted maximum quantity in food products: max. 2000 ppm in dry fruits, grape juice concentrate for home production of wine, in some cases only max. quantities of 20 - 30 ppm are permitted.

For more specific applications, the compounds of the present invention may also be used in combination with the following preservatives: biphenyl, diphenyl, orthophenylphenol, thiabendazol, nisin, natamycin, hexamethylentetramine, dimethyldicarbonate, boric acid, sodiumtetraborate, nitrite, propionic acid and propionate, and lysozyme. The mode of action of these preservatives, together with their range of effects and specific uses are listed below.

Biphenyl, Diphenyl (E 230)

Range of effects: Inhibition of moulds.

Substance for treatment of fruits: surface treatment of citrus fruits.

Permitted maximum quantity: 70 ppm

Orthophenylphenol (E 231 / E 232)

As with E230, limited to treatment of fruits as a surface treatment for citrus fruits.

Thiabendazol (E 233)

Surface treatment of citrus fruits and bananas.

Nisin (E 234)

Mode of action: Disturbance of membrane functions.

Range of effects: Gram-positive bacteria, no influence on Gram-negative bacteria.

Permitted maximum quantity in food products (EU): 3ppm in semolina pudding and similar products, 12.5 ppm (= 12.5 IU/g) in ripened cheese and processed cheese, 10 ppm in clotted cream, 10 ppm in mascarpone.

Natamycin (Pimaricin) (E235)

Mode of action: specifically attacks cell membrane, where - in general - an interaction with sterines occurs which increases the permeability of the membrane.

Range of effects: Moulds and yeasts, not effective against bacteria. Usual dosage rates are below approx. 50 mg / l. Maximum level is 1 mg/dm^2 on the surface, with a maximum penetration of 5 mm.

Applications: surface treatment of hard, semi-hard and semi-soft cheese and of dried, cured sausages.

Hexamethylentetramine (E 239)

Hexamethylentetramine is formed by adding ammonia to formaldehyde in an aqueous solution. The microbicidal effect is due to the formaldehyde.

Permitted only for Provolone cheese (25 ppm residual quantity).

Dimethyldicarbonate (E 242)

Permitted only for non-alcoholic drinks, non-alcoholic wine, and liquid concentrate.

Boric Acid, Sodiumtetraborate (E284 / E 285)

Permitted only for caviar.

Nitrite (E 249 and E 250)

Permitted in the form of nitrite curing salt for treatment of meat products ("red products"). For cured and dried meat products which are not heat treated and for other cured meat products an addition of 150 ppm has been fixed as a guideline. These concentrations do not show a preservative effect. They are mainly added for their technological properties (formation of colour, taste) as well as for their antioxidant effects.

Propionic Acid and Propionate (E 280, E 281, E 282, and E 283)

Mode of action: similar to sorbic acid, pH < 4.5 is optimal.

Accumulation in the cell leads to inhibition of enzymes.

Range of inhibition: moulds are inhibited at an pH of 5.5 by concentrations of 125 to 12500 ppm, for inhibition of bacteria higher concentrations are necessary (> 16000 ppm).

Application: Sliced and packaged bread.

Permitted maximum quantity: 3000 ppm.

Lysozyme (E 1105)

Permitted only for ripened cheese.

Permitted maximum quantity: quantum satis.

Studies by the applicant of the inhibitive effects of the present compounds have been tested in a medium (Elliker broth) with an almost neutral pH (pH 6.8) and have been shown to be effective against both Gram-positive and Gram-negative bacteria. As many of the preservatives described above show an inhibitory effect mainly at low pH, the use of the compounds of the present invention clearly broadens the potential range of applications.

In principle, the use of substances for chemical preservation depends on the following factors:

(a) Toxicological harmlessness

- the effects of the substance when applied acutely, subchronically, and for a long term period.

- Testing of acute toxicity (LD_{50}), cinetics and metabolism, pharmacological effects, genotoxicity, etc.

(b) Technological / food chemical aspects:

- Solubility in water: as growth takes place in the aqueous phase, a preservative has to be water-soluble
- Reaction with food ingredients, problem of off-flavours (sensory acceptance)
- Interferences with food ingredients (e.g. destruction of vitamin B1 by sulphuric acid)

The antimicrobial effectiveness of chemical substances in food and feed products is thus determined by a range of different factors. Among others, the composition of the population of micro-organisms, the composition of the food product (ingredients, pH, water activity, content of salt, etc.), the packaging, time-temperature-conditions, etc. are key factors that influence the inhibitory activities of the antimicrobial agent.

The invention will now be described only by way of example, and with reference to the accompanying figures, wherein:

Figure 1 shows a photograph of well diffusion tests on *M. luteus* (top plate), *B. cereus* (middle two plates), and *Cl. Sporogenes* (bottom two plates) treated with the following:
Upper right segment: 3 % C_8 anhydrofructose ester;
Middle right segment: 0.3 % C_8 anhydrofructose ester;
Lower right segment: 3 % C_{12} anhydrofructose ester;
Lower left segment: 0.3 % C_{12} anhydrofructose ester;
Middle left segment: equivalent methanol control at 25 % methanol;
Upper left segment: equivalent methanol control at 2.5 % methanol.

Figure 2 shows a photograph of a well diffusion test on *M. luteus* treated with the following:
Segment 1: 3 % C_8 anhydrofructose ester;
Segment 2: 0.3 % C_8 anhydrofructose ester;

Segment 3: 3 % C_{12} anhydrofructose ester;

Segment 4: 0.3 % C_{12} anhydrofructose ester;

Segment 5: equivalent methanol control at 25 % methanol;

Segment 6: equivalent methanol control at 2.5 % methanol.

EXAMPLES

CHEMICAL SYNTHESIS

The compounds of the invention were prepared, characterised and purified in accordance with the general methods disclosed in WO 00/56745.

MATERIALS AND METHODS

TEST STRAINS

All microorganisms were taken from storage at -80 °C. Most organisms were tested as vegetative cell suspensions from overnight broth culture. *Bacillus* and *Clostridium* species were tested as endospore suspensions prepared earlier and stored at 4 °C.

For broth cultures and Bioscreen testing most bacteria were grown in Brain Heart Infusion (BHI, Oxoid, pH 7.4). *Lactobacillus sake* A10 was grown in de Man, Rogosa, Sharpe medium (MRS, Oxoid). Yeasts were grown in Sabouraud Liquid medium (SLM, Oxoid). Most bacteria were cultured at 30 °C. Lactic acid bacteria were grown on solid medium in enriched CO_2 atmosphere. *Clostridium* species were grown in Reinforced Clostridial Medium (RCM) at 37 °C anaerobically. *Brochothrix thermosphacta* and yeasts were grown at 25 °C.

Bioscreen testing

An automated Microbiology Reader Bioscreen C was used to measure growth curves of the strains in the presence and absence of test samples. The Bioscreen C measures the development of turbidity (i.e. growth) kinetically by vertical photometry in 200 wells of a honeycomb microtitre plate, simultaneously. The system consists of a Bioscreen C

analyser, which is an incubator and measurement unit, integrated with a PC, software (BioLink v 5.30), printer and a 'Honeycomb 2' cuvette multiwell plate. Growth curve data can be analysed within the BioLink software or exported to programs such as Excel.

Protocol

To a 14 mg sample was added 50 µl of 100% methanol. 66.7 µl of IMS was then added (industrial methylated spirit, 96% ethanol) to make a 12% (w/v) solution.

For the test, this solution was then diluted 1 in 4 in sterile distilled water. This was necessary because the level of alcohol in the sample would otherwise be inhibitory to the test micro-organism. This made a final solution of 3% (w/v).

The test sample could not be filter sterilised because too much would have been lost, and only ca. 470 µl was available. The sample had been handled aseptically and it was hoped that it was sterile. For the same reason the pH of the sample was not measured.

The sample was then tested at 0.3% concentration in the Bioscreen. However it was immediately realised that this may be problematic because the AF-ester 1 test sample was milky-white and turbid. Unfortunately, when this was added to the Bioscreen wells, the initial turbidity was too high for any microbial growth to be discerned. Therefore to ascertain if any inhibition had occurred, viable counts were taken of the inoculum, and then after 24 h incubation in the Bioscreen at 30 °C, by sampling directly from the Bioscreen plate. Inhibition could then be assessed by comparison with the final numbers achieved in the control wells that contained 2.5% alcohol.

Results of Bioscreen BS021100

AF ester 1 = C_8 ester of anhydrofructose (structure shown in claim 32 - LHS).

Table 1

Test strain	Initial count (cfu/ml)	Count after 24 h at 30 °C (cfu/ml)	
		2.5% alcohol control	0.3% AF-ester 1
B. cereus 204	1×10^3	1.3×10^7	2.4×10^2
L. monocytogenes S23	1.2×10^3	1.1×10^9	$< 10^2$
Lb. sake A10	$< 10^2$	1.9×10^2	$< 10^2$
E. coli S15	5.3×10^2	1.1×10^9	3.7×10^7
Ps. fluorescens 3756	3.6×10^2	1.1×10^9	2.3×10^7
S. cerevisiae 9763	3.6×10^2	2.0×10^6	2×10^1
S. carlsbergensis 6418	5.2×10^2	9.7×10^4	1×10^1

Conclusions

The results in Table 1 show that AF-ester 1 was inhibitory towards all the micro-organisms tested. The order of inhibitory activity was as follows: Gram positives > yeasts > Gram negatives. The sample was particularly effective against *L. monocytogenes*, but was also very effective against *Bacillus*. There was evidence of cidal activity towards *L. monocytogenes*, and possibly the yeasts.

Anhydrofructose ester 1: Cidal test

A preliminary cidal experiment was undertaken with the sample that had earlier been tested in Bioscreen with viable count confirmation. This had shown good activity. For the cidal experiment the chosen test organism was *L. monocytogenes* S23, because this had shown the greatest sensitivity in the growth inhibition testing.

Protocol

Aliquot 3 x 890 ml 10 mM HEPES buffer, pH 7. To the control test was added 100 ml water, to the other control test was added 100 ml equivalent alcohol control and to the test sample was added 100 ml AF ester 1. To all tests were added 10 ml of an overnight culture. The samples were left at ambient temperature for 2 h. A viable count was carried out. Note: the AF ester 1 precipitated out during the test.

Results

Tests	Viable count (cfu/ml)
Control/water	3.1×10^8
Control/alcohol	2.0×10^7
Test/ AF ester 1	2.4×10^7

From the results it was concluded that AF ester 1 does not have any cidal activity.

Testing of new samples:

Anhydrofructose ester C8 (AFC8) = C_8 ester of anhydrofructose (structure shown in claim 32 - LHS)

Anhydrofructose ester C12 (AFC12) = C_{12} ester of anhydrofructose (structure shown in claim 32 - RHS)

Glucose ester C8 (GC8) - control

Glucose ester C12 (GC12) - control

AF esters were dissolved in water by either heating at 70 °C for 10 – 15 min, or 100 °C for 5-10 minutes. Both methods were unsuccessful, and the esters were eventually tested as 0.5 % (w/v) solutions in 50:50 methanol/water that had been heated. AFC8 did not dissolve, but the others were better.

Results:

No zones observed for equivalent methanol controls.

Table 2

Test strain	Well diffusion zone (mm) tested against 0.5% (wt/vol) extracts			
	AF C8	AF C12	Glucose C8	Glucose C12
B. cereus 204	0	6.82	0	0
Cl. sporogenes Campden	3.90	11.30	0	+/- (3.50)
L. monocytogenes S23	0	0	0	0
Lb. sake A10	0	0	0	0
Br. thermosphacta CRA7883	0	7.50	0	0
Micrococcus luteus	0	8.95	0	0
E. coli S15	0	0	0	0

Ps. fluorescens 327	0	0	0	0
S. cerevisiae ATCC 9763	0	+/- (10.00)	0	0
S. carlsbergensis CRA6413	0	0	0	0

Results of Bioscreen run BS191200

Table 3

Strain (Control: average OD without methanol)	Level tested % (wt/vol)	Final – min OD for 18 or 24 h growth at 30 °C				
		AFC8	AFC12	GC8	GC12	Control: Equivalent Methanol level
B. cereus 204 (0.80)	0.05	0	0	0.242	0	0.194
	0.025*	0.168	0	0.785	0.561	0.619
B. cereus Campden (0.72)	0.05	0	0	0.882	0.437	0.75
	0.025*	0.8	0	0.777	0.641	0.707
L. monocytogenes S23 (0.66)	0.05	0	0	0.242	0.19	0.27
	0.025	0.027	0	0.514	0.379	0.537
Lb. sake A10 (0.90)	0.05	0	0	0.245	0.392	0.123
	0.025	0.535	0.376	0.81	0.785	0.477
E. coli S15 (0.95)	0.05	0.573	0.478	0.654	0.745	0.716
	0.025	0.753	0.703	0.818	0.875	0.851
E. coli CRA109 (0.86)	0.05	0	0	0.285	0.149	0.187
	0.025	0.697	0.738	0.789	0.759	0.84
Ps. fluorescens 3756 (1.2)	0.05	0	0	0	0	0
	0.025	0.068	0.192	0.82	0.953	0.973
Ps. fluorescens 327 (0.37)	0.05	0	0	0	0	0
	0.025	0.12	0.178	0.21	0.354	0.219

*AF C12 showed total inhibition of Bc204 and Bc Campden at a minimum level tested of 0.0125%.

Controls for this run were based on the final – OD for 18 or 24 h growth at 30 °C for growth in equivalent methanol levels. Inhibition by the AF esters was judged by whether the number was lower than the number derived for the methanol control.

CONCLUSIONS

- The well diffusion results showed that 0.5% AFC8 and AFC12 both had anti-clostridial activity, and AFC12 had activity against *Bacillus*, *Brochothrix*, *Micrococcus* and perhaps yeasts, but not *L. monocytogenes*, or gram negatives (GN).

- Bioscreen results were from tests with 0.05% samples.
- Bioscreen confirmed the order of activity was as follows: AFC12 > AFC8. Bioscreen also confirmed activity against *Bacillus*, but activity was also observed against *L. monocytogenes*, and *Lb sake*, as well as some activity against gram negatives (GN).

Description of Bioscreen analysis: BS040101

0.3% AF ester was made up in 2.5% methanol. Serial dilutions were made. The following concentrations were tested: 0.3, 0.15, 0.075, 0.038 and 0%. APP was made up in water. The samples were analysed after 24 h at 30 °C (Table 4).

Table 4

Strain	AF ester C8	AF ester C12
Bc 204	Total inhibition at 0.15% Inhibition to 0.075%	Total inhibition at 0.038%
Lm S23	Total inhibition at 0.15% Inhibition to 0.038%	Total inhibition at 0.038%
Lbs A10	Total inhibition at 0.3% Inhibition to 0.15%	Total inhibition at 0.15% Inhibition to 0.038%
Ec S15	No inhibition at 0.3%	No inhibition at 0.3%
Psf 3756	Inhibition to 0.3%	Inhibition to 0.3%

Viable counts from BS040101

Inhibition was judged by whether the final count in the presence of either AF ester was lower than the final count in 2.5 % methanol (control). The results are shown in Table 5.

Table 5

	Final viable count after 24 h (cfu/ml)		
Strain	0.3% AFE C8	0.3% AFE C12	2.5% methanol control
Bc 204	1×10^3	1.5×10^3	1×10^5
Lm S23	2.5×10^3	1.5×10^3	2.9×10^9
Lbs A10	$< 10^5$	$< 10^5$	3.7×10^7
Ec S15	3.5×10^7	1.7×10^9	2.3×10^9
Psf 3756	1.7×10^8	2.7×10^8	3.2×10^9
Sce 9763	9.4×10^4	7.4×10^2	4.5×10^6
Sca 6413	3.3×10^4	nd	1.4×10^4

Well diffusion testing

The results for *M. luteus*, *B. cereus* 204, *B. cereus* Campden, *Cl. sporogenes* 1.221, and *Cl. sporogenes* Campden are illustrated in Figures 1 and 2 and Table 6. None of the methanol control tests gave any diffusion zones. Code for the wells: 1 = 3% AFEC8, 2 = 0.3% AFEC8, 3 = 3% AFEC12, 4 = 0.3% AFEC12, 5 = equivalent methanol control at 25% methanol, 6 = equivalent methanol control at 2.5% methanol.

Table 6

Test strain	Well diffusion zone (mm) tested against 0.3% and 3% (wt/vol) extracts			
	AFE C8 3%	AFE C8 0.3%	AFE C12 3%	AFE C12 0.3%
B. cereus 204	2.29	0	7.75	1.27
B cereus Campden	< 0.5	0	3.23	< 0.5
Cl sporogenes 1.221	6.00	0	16.83	5.06
Cl. sporogenes Campden	6.15	5.05	14.15	6.15
L. monocytogenes S23	1.62	0	2.26	+/-
L. monocytogenes 272	2.36	0	+/- 8.82	0
Lb. sake A10	+/-	0	+/- (2.8)	+/-
Br. thermosphacta CRA7883	0.98	0	7.55	+/-
Micrococcus luteus	3.95	0	9.42	2.33
E. coli S15	0	0	0	0
Ps. fluorescens 327	0	0	0	0
Ps fluorescens 3756	0	0	0	0
S. cerevisiae ATCC 9763	E	E	+	E
S. carlsbergensis CRA6413	E	E	+	E

CODE (for yeasts): E = enhanced growth; + = zone of inhibition observed.

Further testing of APP

Well diffusion zones at 3% vs *Cl. sporogenes* Campden (4.72) and *Br. thermosphacta* 7883 (2.96)

Viable counts of BS040101

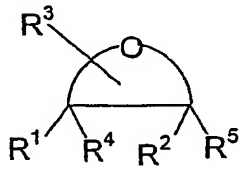
Table 7

Strain	Viable count in 0.3% APP
Bc 204	7.0×10^{1}
Lm S23	5.4×10^{7}
Lbs A10	1.9×10^{8}
Ec S15	6.2×10^{8}
Psf 3756	$< 10^{2}$
Sce 9763	3.1×10^{5}
Sca	1.1×10^{5}

Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Modifications of the described modes for carrying out the invention which are obvious to those skilled in the relevant are, or related fields, are thus intended to fall within the scope of the following claims.

CLAIMS

1. An antimicrobial composition comprising a cyclic compound having Formula I,



I

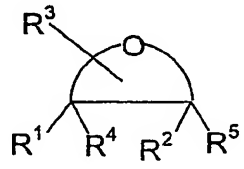
wherein R^1 and R^2 are independently selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group

wherein R^3 is selected from -OH, =O, a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group;

wherein R^4 and R^5 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound;

and wherein said compound comprises at least one ester group.

2. A process for preventing and/or inhibiting the growth of, and/or killing, microorganisms in a material, the process comprising the step of contacting the material with a cyclic compound having Formula I,



I

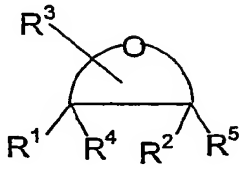
wherein R^1 and R^2 are independently selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group

wherein R^3 is selected from -OH, =O, a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group;

wherein R^4 and R^5 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound;

and wherein said compound comprises at least one ester group.

3.Use of a compound having Formula I,



I

wherein R^1 and R^2 are independently selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group

wherein R^3 is selected from -OH, =O, a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group;

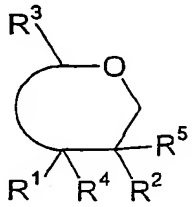
wherein R^4 and R^5 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound;

and wherein said compound comprises at least one ester group;

for preventing and/or inhibiting the growth of, and/or killing, microorganisms in a material.

4. The invention according to any one of the preceding claims wherein said material is a foodstuff or feed.

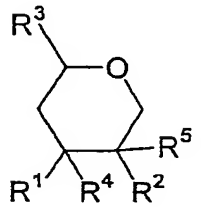
5. The invention of any one of the preceding claims wherein the cyclic compound is a compound having Formula II



II

wherein R^1, R^2, R^3, R^4, and R^5 are as defined in the preceding claims.

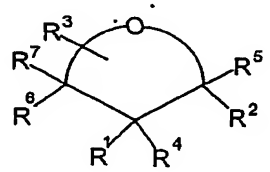
6. The invention of any one of the preceding claims wherein the cyclic compound is a compound having Formula III



III

wherein R^1, R^2, R^3, R^4, and R^5 are as defined in the preceding claims;

7. The invention of any one of the preceding claims wherein said cyclic compound is of Formula IV,



IV

wherein R^1 and R^2 are independently selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group

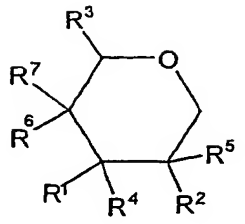
wherein R^3 is selected from -OH, =O, a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group;

wherein R^4 and R^5 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound;

wherein R^6 and R^7 are each independently selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group or wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound;

and wherein said compound comprises at least one ester group.

8. The invention of any one of the preceding claims wherein said cyclic compound is of formula V,



V

wherein R^1, R^2, R^3, R^4, R^5, R^6 and R^7 are as defined in claim 7;

9. The invention of any one of the preceding claims wherein R^1 is selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group

10. The invention of any one of the preceding claims wherein R^2 is selected from -OH, =O, and -OC(O)R', wherein R' is a hydrocarbyl group

11. The invention of any one of the preceding claims wherein R^3 is selected from a substituent comprising an -OH group and -OC(O)R', wherein R' is a H or a hydrocarbyl group;

12. The invention of any one of the preceding claims wherein R^3 is -OC(O)R', wherein R' is a H or a hydrocarbyl group;

13. The invention of any one of the preceding claims wherein R^3 is -OC(O)R', wherein R' is a hydrocarbyl group;

13. The invention of any one of the preceding claims wherein R^3 is -OC(O)R', wherein R' is R'' group;

14. The invention of any one of the preceding claims wherein R' and/or R'' is a branched or unbranched, substituted or unsubstituted alkyl group.

15. The invention of any one of the preceding claims wherein R' and/or R'' is $(CH_2)_pCH_3$, wherein p is from 1 to 24.

16. The invention of any one of the preceding claims wherein R' and/or R'' is a C_8 alkyl group.

17. The invention of any one of the preceding claims wherein R' and/or R'' is a C_{12} alkyl group.

18. The invention according to any one of the preceding claims R^3 is of the formula -$(CH_2)_n$-OC(O)-$(CH_2)_pCH_3$, wherein n and p are each independently from 1 to 24.

19. The invention according to any one of the preceding claims R^3 is of the formula -$(CH_2)_n$-OC(O)-$(CH_2)_7CH_3$, wherein n and p are each independently from 1 to 24.

20. The invention according to any one of the preceding claims R^3 is of the formula -$(CH_2)_n$-OC(O)-$(CH_2)_{11}CH_3$, wherein n and p are each independently from 1 to 24.

21. The invention of any one of the preceding claims wherein R^4 is selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group.

22. The invention of any one of the preceding claims wherein R^4 is selected from a hydrocarbyl group, H, OH, and =O.

23. The invention of any one of the preceding claims wherein R^5 is selected from a hydrocarbyl group, H, OH, =O, and -OC(O)R', wherein R' is a H or a hydrocarbyl group.

24. The invention of any one of the preceding claims wherein R^5 is selected from a hydrocarbyl group, H, OH, and =O.

25. The invention of any one of the preceding claims wherein R^4 and R^5 represent a bond with an adjacent atom on the ring of the cyclic compound;

25. The invention of any one of the preceding claims wherein the compound is esterified.

anhydrofructose wherein at least one OH group of anhydrofructose is esterified to form a -OC(O)R''' group, wherein R''' is a hydrocarbyl group.

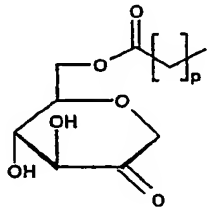
26. The invention of claim 25 wherein R''' is a branched or unbranched, substituted or unsubstituted alkyl group.

27. The invention of claim 25 wherein R''' is $(CH_2)_pCH_3$, wherein p is from 1 to 24.

28. The invention of claim 25 wherein R''' is a C_8 alkyl group.

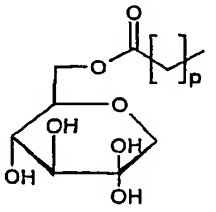
29. The invention of claim 25 wherein R''' is a C_{12} alkyl group

30. The invention of any one of the preceding claims wherein the cyclic compound is of the formula:



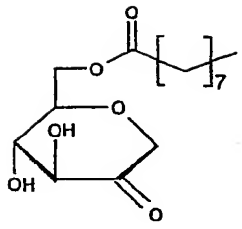
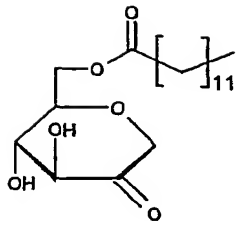
p = 1-24

31. The invention of any one of the preceding claims wherein the cyclic compound is of the formula:

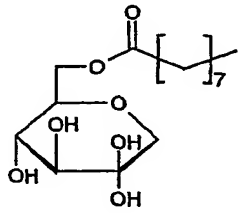
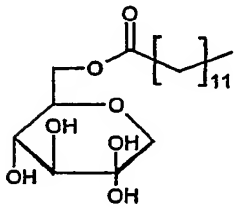


p = 1-24

32. The invention of any one of the preceding claims wherein said cyclic compound is selected from the following:

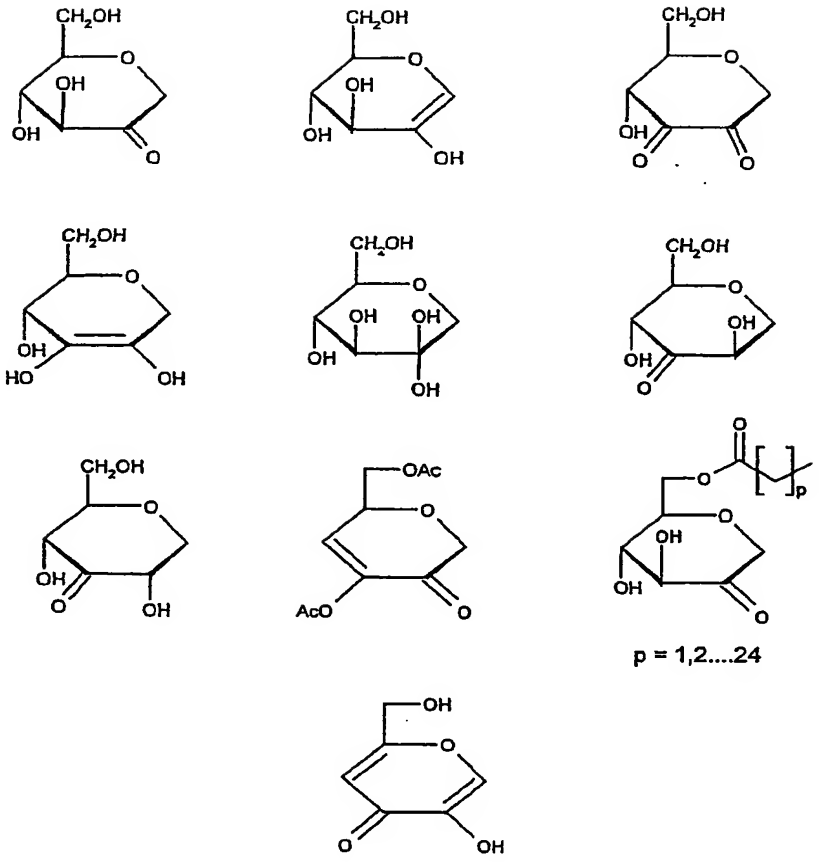
33. The invention of any one of the preceding claims wherein said cyclic compound is selected from the following:

34. The invention of any one of the preceding claims wherein the compound is a derivative of Ascopyrone P, Ascopyrone M, Ascopyrone T, Ascopyrone T_1, Ascopyrone T_2, Ascopyrone T_3, and mixtures thereof.

35. The invention of any one of the preceding claims wherein the compound is selected from esterified Ascopyrone P, esterified Ascopyrone M, esterified Ascopyrone T, esterified Ascopyrone T_1, esterified Ascopyrone T_2, esterified Ascopyrone T_3, and mixtures thereof.

36. The invention of any one of the preceding claims wherein the compound is selected from the following:



or an esterified derivative thereof.

37. The invention of any one of the preceding claims wherein the cyclic compound having formula I has an antimicrobial effect against a microorganism selected from *Listeria, Salmonella, Bacillus, Saccharomyces, Pseudomonas, Clostridium, Lactobacillus, Brochothrix, Micrococcus, Yersinia, Enterobacter* and *Zygosaccharomyces, Staphylococcus,* and *Escherichia.*

38. The invention of any one of the preceding claims wherein the cyclic compound having formula I has an antimicrobial effect against a microorganism selected from *Listeria monocytogenes, E. coli, Staphylococcus aureus, Listeria innocua, Salmonella* Typhimurium, *Salmonella* sp., *Bacillus cereus, Bacillus subtilis, Saccharomyces cerevisiae, Saccharomyces cerevisiae* var. *paradoxus, Saccharomyces carlsbergensis, Pseudomonas fluorescens, Clostridium sporogenes, Lactobacillus sake, Brochothrix thermosphacta, Micrococcus*

luteus, Yersinia enterocolitica, Enterobacter aerogenes and *Zygosaccharomyces bailii.*

39. The invention of any one of the preceding claims wherein the cyclic compound having formula I has an antimicrobial effect against a micro-organism selected from *Listeria monocytogenes, E. coli, Bacillus cereus, Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Pseudomonas fluorescens, Clostridium sporogenes, Lactobacillus sake, Brochothrix thermosphacta* and *Micrococcus luteus.*

40. The invention of any one of the preceding claims wherein said compound of formula I is used in combination with one or more of an antioxidant, a preservative and/or a chelator.

FIGURE 1

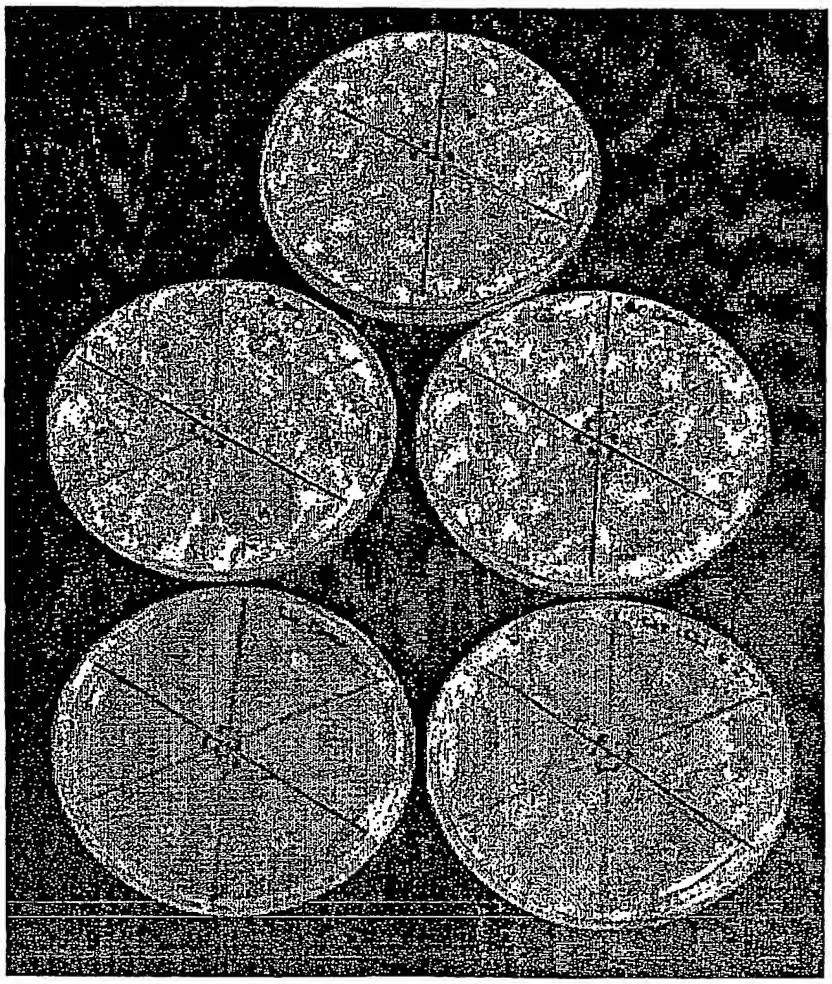
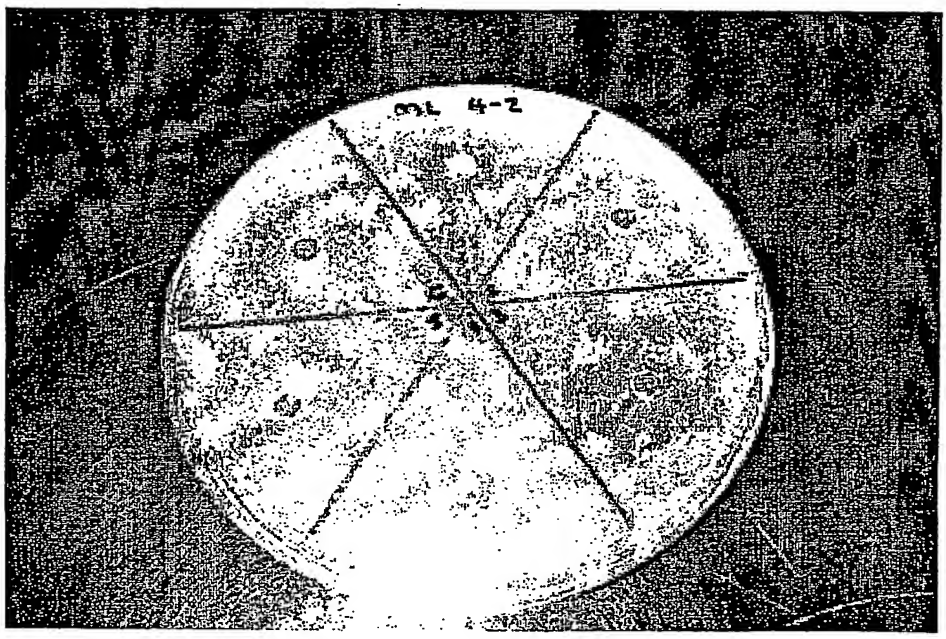


FIGURE 2



INTERNATIONAL SEARCH REPORT

In ial Application No
PCT/GB 01/04330

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L3/3562 A23L3/3544 A61L2/16 A61L2/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
FSTA, EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	WO 95 10616 A (DANISCO) 20 April 1995 (1995-04-20) claims 1,21,22; examples 4-1,4-2	1-6, 34-40
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 January 2002	08/02/2002

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

In ...nal Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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X,P	WO 00 56745 A (ANDERSEN SOEREN MOELLER ;DANISCO (DK); MARCUSSEN JAN (DK); LUNDT I) 28 September 2000 (2000-09-28) page 11, line 7 - line 26; claims page 5, line 11 -page 9, line 25	1-40
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A	GB 2 358 137 A (DANISCO) 18 July 2001 (2001-07-18) page 21, line 7-10; claims 1-12 page 13, line 9 -page 16, line 2	1-40
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A	FR 2 617 502 A (ELF AQUITAINE) 6 January 1989 (1989-01-06)	

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